Attachment R
Fresh Water Acute Toxicity Test Procedure and Protocol

- Daphnids (Ceriodaphnia dubia) definitive 24 hour acute test.
- Fathead Minnow (Pimephales promelas) definitive 24 hour acute test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Acute toxicity test data shall be reported as outlined in Section IX.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

An acute toxicity test sampling event is defined as a single discharge (composite or grab) sample.

III. METHODS

Methods should follow those recommended by EPA in:

Peltier, W., and Weber, C.I., 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater, Third Edition. Office of Research and Development, Cincinnati, OH. EPA/600/4-90-027 Rev. 9/91 Section 6.1.).

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for chemical and physical analyses required. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual chlorine (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. REGION I RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR THE DAPHNIDS (Ceriodaphnia dubia and) 24 HOUR ACUTE TESTS[1]

1. Test type	Static, non-renewal
2. Temperature (°C)	25 ± 1°C
3. Light quality	Ambient laboratory illumination
4. Photoperiod	16 hour light, 8 hour dark
5. Test chamber size	Minimum 30 ml

6. Test solution volume	Minimum 25 ml
7. Age of test organisms	1-24 hours (neonates)
8. No. daphnids per test chamber	5
9. No. of replicate test chambers per treatment	4
10. Total no. daphnids per test concentration	20
11. Feeding regime	None
12. Aeration	None
13. Number of dilutions	1 plus a control.
14. Effect measured	Mortality - no movement of body or appendages on gentle prodding
15. Test acceptability	90% or greater survival of test organisms in control solution
16. Sampling requirements	For on-site tests, samples must be used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must first be used within 24 hours of collection.
17. Sample volume required	Minimum 2 liters

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. Standard prepared dilution water must have hardness requirements to generally reflect the characteristics of the receiving water.

VI. REGION I RECOMMENDED TEST CONDITIONS FOR THE FATHEAD MINNOW (Pimephales promelas) 24 HOUR ACUTE TEST[1]

1.	Test Type:	Static, non-renewal
2.	Temperature (^{o}C):	25 $\pm$ 1^{o}C
3.	Light quality:	Ambient laboratory illumination
4.	Photoperiod:	16 hr light, 8 hr dark
5.	Size of test vessels:	250-1000 ml
6.	Volume of test solution:	Minimum 200ml/replicate
7.	Age of fish:	1-14 days

8.	No. of fish per chamber:	10 (not to exceed loading limits)
9.	No. of replicate test vessels per treatment:	2
10.	Total no. organisms per concentration:	20
11.	Feeding regime:	None
12.	Aeration:	None, unless DO concentration falls below 40% of saturation, at which time gentle single-bubble aeration should be started at a rate of less than 100 bubbles/min. (Routine DO check recommended.)
13.	Number of dilutions:	1 plus a control.
14.	Effect measured:	Mortality-no movement on gentle prodding
15.	Test acceptability:	90% or greater survival of test organisms in control solution
16.	Sampling requirements:	For on-site tests, samples must be used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.
17.	Sample volume required:	Minimum 4 liters

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Footnotes:

1. Adapted from EPA/600/4-85/013.
2. Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.

VII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

Parameter			Minimum Detection Effluent Diluent Limit (mg/L)
Hardness[*1]	x	x	0.5
Alkalinity	x	x	2.0
pH	x	x	---
Specific Conductance	x	x	---
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5
Total Residual Chlorine (TRC)[*2]	x	x	0.02

Total Metals

Cd	x		0.005
Cr, Ni	x		0.05
Pb, Cu	x	x	0.005
Zn, Al	x	x	0.02
Mg, Ca	x	x	0.05

Superscripts:

[*1] Method 314A (hardness by calculation) from APHA (1985) Standard Methods for the Examination of Water and Wastewater. 16th Edition.

[*2] Total Residual Chlorine

Methods: either of the following methods the 16th edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses.

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

VIII. TOXICITY TEST REPORT

The following must be reported:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Survival for each concentration and replication at time 24 hours.
- LC50 and 95% confidence limits shall be calculated using one of the following methods in order of preference Probit, Trimmed Spearman Karber, Moving Average Angle, or the graphical method. All printouts (along with the name of the program, the date, and the author(s)) and graphical displays must be submitted. When data is analyzed by hand, worksheets should be submitted.

  The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.
- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets.
- Describe method of dechlorination where applicable.
- Any observations and test conditions which affected the outcome of testing.

IX. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by of Part I of the NPDES permit.

- July 1990 -